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THE DETERMINATION OF PHENOLIC MATERIALS IN INDUSTRIAL WASTES

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The Determination of Phenolic Materials in Industrial Wastes

In this consideration of the determination of phenolic materials in industrial wastes, the discussion will be limited almost entirely to methods and procedures which the authors have actually used. No effort will be made to present an extensive literature survey or to catalogue the multitude of methods which have been proposed for determining various phenolic materials. Treatment of the problem from this limited viewpoint will obviously cause much material to go unmentioned. However, the narrow approach chosen has the advantage of permitting detailed examination of the techniques treated, and some discussion of the interpretation of results secured through using those techniques.

To the organic chemist, a "phenol" is a compound which has at least one hydroxyl group attached directly to a benzene ring. This definition is quite general and inclusive. However, from the standpoint of the analysis of industrial wastes, such a general definition of a "phenol", or phenolic material, is rarely applied.

It is an operational fact that the definition of "phenol" for the purpose of analysis of industrial wastes hinges upon the technique used. The method selected actually determines what

is classed as "phenol". In a particular case, "phenol" is defined simply as the material recovered and reported as "phenol" after the prescribed method has been applied.

From the sanitary standpoint, when a "phenol" analysis is made on an industrial waste, it is desired to make collective determination of a fairly limited part of those compounds which would be classed as "phenols" by the organic chemist. The compounds actually desired are monohydric phenols, that is, phenols which include only one hydroxyl group per benzene ring in their structure. In most cases, it is also desired to limit the determination to fairly simple phenolic compounds which have their chemical properties largely dominated by the fact that they include in their structure a benzene ring substituted by one hydroxyl group. This group includes materials such as phenol, the cresols, the xylenols, the naphthols, etc. In general, it is not desired to determine with the phenol group, materials such as picric acid, various tannins or the like.

In practice, the procedures used will rarely determine as "phenol" materials which are highly substituted and which no longer have their chemical properties largely dominated by the fact that they include in their structure one hydroxyl group attached to a benzene ring. Existing procedures leave much to be desired when they are considered as definitions of "phenol".

Unfortunately there is little prospect of the development of procedures suitable for routine use which give results corresponding to the exact portion of the "phenol group" generally desired.

Phenol procedures for industrial wastes consist of two phases. First, the waste is put through a screening procedure to separate the phenolic constituents from many of the other components of the waste. Next, a measuring reaction is applied, or a physical characteristic determined, to permit estimation of the amount of "phenol" present.

"Phenol" Screening Procedures for Industrial Wastes

A screening procedure should eliminate those constituents of the waste which will give either positive or negative interference with the method used to estimate the amount of "phenol" present. Before any screening procedure can be considered for incorporation into a "phenol" determination, it must be established that none of the "phenol" is lost by the process. Fractional recovery of the phenolic constituents is usually sufficient cause to preclude usage of a screening procedure.

In general, screening procedures used in "phenol" determinations consist of one or more of the following operations:

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(1) Distillations of various types.

(2) Alkaline evaporation.

(3) Treatment with a precipitant for an interfering material.

(4) Extraction of the "phenol" from acidified solutions with an organic solvent, with subsequent extraction of the "phenol" from the solvent with an aqueous caustic solution.

Phenols may be distilled from highly acid solutions, from neutral solutions, or from very slightly alkaline solutions with quantitative recovery of many of the "phenols". Boshgatoor, et al (1) carry out certain purifications of phenolic wastes by distillation first from solutions acidified with either sulfuric or phosphoric acid, followed by a redistillation of the distillate with marble chips added. It is interesting to note that the second distillation was used to remove salicylic acid, itself a phenol in the sense that it contains a hydroxyl group attached to a benzene ring.

Distillation of phenol from low pH solutions will accomplish several purposes. Materials screened out include nitrogen bases and nonvolatile inorganic materials. Organic material of high boiling point will be partially or completely left behind.

Materials of phenolic nature also may not distill over quantitatively, especially if they have high boiling points. For instance, 1-naphthol and 2-naphthol will not be distilled over quantitatively as the result of a normal distillation. In practice, recovery of these chemicals by distillation has been observed to be variable and highly incomplete. Beshgetoor et al (1) recommend distillation from sulfuric acid solution if aniline or extremely weak bases are present, otherwise phosphoric acid is the acid of choice.

In addition to the volatile phenols, the distillate from an acid distillation may contain a number of organic compounds. These include volatile neutral compounds and volatile acids.

If copper sulfate is added to the solution before distillation, the screening is further improved. Hydrogen sulfide does not come over in the distillate, even if sulfides are present. In some measure, cyanide may tend to be held back as a copper complex. In general, materials which form extremely insoluble copper salts will either be reduced in amount, or kept out of the distillate altogether.

A type of distillation which has been found useful at the Environmental Health Center is the redistillation of the acid distillate with only copper sulphate added. In particular, this type of distillation has been found to be an effective

step in the determination of phenol in sewage. In the above case, the distillate resulting from an acid distillation with copper sulfate was quite turbid, presumably due to the presence of fatty acids. However, a second distillation with copper sulphate added to the solution resulted in a clear distillate.

Alkaline evaporation is a screening procedure of some value in separating phenolic material from other constituents. Materials which may be distilled out include volatile neutral compounds and nitrogen bases.

This operation has been recommended as a means of concentrating trace quantities of "phenol" (2). However, it frequently happens that substantial amounts of "phenol" are lost in the application of this procedure. Accordingly, the process should be used with caution and the step should not be applied until other purification steps have been completed and many of the materials initially present have been screened out. In particular, this process is used at the Environmental Health Center to remove small amounts of solvent dissolved in water as the result of an extraction separation.

Another process which has been applied is the treatment of phenol solutions with a precipitant for interfering materials,

followed by filtration. For instance, lead, copper and cadmium salts have frequently been used to effect the removal of sulfides. The Environmental Health Center has not recently used a process of this nature. For the removal of sulfides the addition of copper salts to a distillation mixture has been employed.

Extraction and reverse extraction is another general type of screening procedure for phenols. First, an acid solution of the waste is extracted with an immiscible organic solvent. The phenol is separated into the solvent phase. Serial extraction may be required to obtain a quantitative recovery of the "phenol" present. In the case of some solvents, it may be necessary to add sodium chloride or calcium chloride to the water phase in order to make the extraction quantitative. Once the "phenol" is quantitatively transferred to the organic phase, the aqueous phase is discarded. Next, the organic phase is extracted with a dilute solution of sodium hydroxide to yield a water phase which contains sodium phenate or similar compounds which are relatively insoluble in the organic solvent.

At the Environmental Health Center, copper salts are routinely added to a material to be subjected to the extraction purification before the process is started. The addition of

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this material increases the number of substances which can be screened out by the procedure. Cyanides fall in this category. We apply separations of this type using both ether and chloroform as the organic solvent. If it is not necessary to eliminate the solvent subsequently and completely, chloroform is to be preferred over the ether because it is easier to handle. However, when chloroform is used it is necessary to add salt to the water phase when transferring the "phenol" to the organic solvent.

The combination of the two extractions is a very good screening procedure. It will separate phenol from organic bases, many inorganic materials which are not soluble in the organic solvent and many of the neutral organic compounds. The addition of the copper salts is recommended, as it requires little effort and may definitely increase the value of the process. For the examination of most wastes it is desirable to include a double extraction in the procedure.

Determination of the Phenolic Material by Bromine Demand Measurement

When 10 p.p.m. or more of "phenol" is present in a waste it is frequently determined by bromination. Phenol readily takes up bromine with the formation of tribromophenol (3).

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Six atoms of bromine are used up by one molecule of phenol. The cresols also brominate readily and the reaction may be controlled (3) so that six atoms of bromine will be used as in the case of phenol.

When less than 10 p.p.m. of "phenol" is present, it is frequently possible to effect a quantitative concentration of the "phenol" and still apply this procedure. The scrubbing procedures involving double extractions may be adapted to produce five-fold or ten-fold concentrations of "phenol" with relative ease. It is, therefore, quite feasible to adapt this method for use on wastes which contain 1 p.p.m. of "phenol".

The determination of "phenol" by bromination is essentially a bromine demand test. Any non-phenolic material, which has a bromine demand in acid solution, and which is still present when the bromination is carried out, will cause a positive interference with the test. Some of the materials which would have this effect are aromatic amines, unsaturated compounds, inorganic sulphur compounds such as hydrogen sulfide or sulphur dioxide, cyanide and many other inorganic reductants.

Materials which would give negative interference with this test are those which will liberate iodine from iodide in acid solution. These are likely to occur infrequently in industrial wastes.

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Table I.

Reactions of Some "Phenols" When Subject to Scott's Bromination Procedure

Phenolic Material	1			2	Source of data
	10 p.p.m.	20 p.p.m.	30 p.p.m.		
phenol	6	6	6	5.1	Ref.(3)
o-cresol	6	6	6	4.4	Ref.(3)
m-cresol	6	6	6	4.4	Ref.(3)
p-cresol	6	6	6	4.4	Ref.(3)
4 Hydroxy-1,3-dimethyl benzene	4.4	4.5	4.3	2.9	EHC Lab.*
4 Hydroxy-1,2-dimethyl benzene	6.4	6.5	6.5	4.3	EHC Lab.
5 Hydroxy-1,3-dimethyl benzene	6.4	6.3	6.3	4.2	EHC Lab.
1-naphthol	7.2	7.0	6.1	4.0	EHC Lab.
2-naphthol	6.8	6.3	7.2	4.0	EHC Lab.
Thymol	6.4	6.4	6.5	3.8	EHC Lab.

1. No. of bromine atoms required to react with one molecule of the phenolic material in question.

2. Grams of bromine reacted with by 1 gram of the phenolic material, based upon the maximum bromine consumption noted.

* The E.H.C. values are based upon duplicate observations only.

Before making a determination of "phenol" by bromination, it is necessary to apply screening procedures which will separate the phenolic material from interfering materials present in the waste.

When put through Scott's procedure (3), the amount of bromine with which a given phenolic material will react is difficult to predict accurately. In Table I are listed Scott's observations regarding phenol and the cresols as well as some supplementary data.

Scott's procedure is highly empirical in nature. The control of many variables in the reaction is closely specified. In the case of some materials, relatively large deviations from the procedure make little difference in the results obtained. This condition, unfortunately, does not generally prevail. Accordingly, in order to achieve a high degree of reproducibility or interchangibility of data, it is necessary to follow a closely controlled procedure such as Scott describes.

The interpretation of the results obtained by bromination is not always easy. When only one phenolic material is present, the calculation and presentation of results is well defined. In such a case, the bromine consumption may readily be calculated in terms of the particular material involved.

However, if a complex mixture of phenolic materials is analyzed by this method, it is impossible to ascribe to the results a definite significance in terms of the total concentration of phenolic materials present. However, there are no common phenolic materials which will react with as much bromine per unit weight as will phenol (the specific compound). Hence, when a mixture is examined and the specific phenolic constituents and their proportions are not definitely known, it is customary to report the results as "phenol". When so reported, a value is indicative of the minimum amount of phenolic material which could be present.

As an example of the application of this procedure we have used this method in the examination of some by-product coke wastes. First the phenolic material is distilled from an acid solution containing copper sulphate. Salt, acid and copper sulphate are then added to the distillate and it is extracted three times with chloroform. The chloroform extract is then extracted twice with dilute alkali. The alkaline extract may then be used to make a bromine demand test. The bromine demand present, usually calculated as phenol, is used as the basis for the determination of phenolic material. This procedure is given in detail in appendix 1.

The Gibbs Procedure

This procedure finds its best field of usefulness in the determination of trace amounts of certain phenolic materials. If standards or objectives for industrial waste quality permit the discharge of relatively minute amounts of phenol, this method may be specified as the means employed to determine whether a particular waste complies with the standards. For instance, in the "Objectives for Boundary Water Control", the International Joint Commission specified the use of a Gibbs procedure (4) to determine whether wastes comply with the objectives, (20 p.p.b., or less of phenol was suggested as the limit).

The Gibbs reaction consists of the addition of 2,6 - dibromquinonechlorimide to a somewhat alkaline solution of the phenolic material being examined. In order to secure good quantitative results, the conditions under which the reaction is carried out must be carefully specified and controlled (6). While the color developed by the reaction may be observed directly (8), it is current practice to extract and concentrate the dye using an organic solvent (5) (1) (6). In general, this reaction cannot be satisfactorily applied to industrial wastes without the use of a preliminary screening procedure. This is

especially true if the "phenol" constitutes a relatively small amount of the organic matter present in the waste.

Current practice at this laboratory as regards the application of the Gibbs reaction to river waters has already been described in some detail (6). Screening procedures suitable for adapting the procedure to industrial wastes on a fairly universal basis have also been presented (4)*. Accordingly, this discussion will not directly describe the details of procedures. Instead, some of the difficulties which may be encountered in carrying out the Gibbs reaction will be treated, along with a consideration of remedies which have frequently been successful.

If "phenol" is known to be present and no color develops, a number of factors may be responsible. These include:

(1) Failure to adjust the reaction mixture to the proper pH.

(2) Failure to screen out sulfides or other powerful reducing agents. (The dye produced is a redox indicator (9) and is affected by reducing agents).

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* The I.J.C. "Phenol" procedure is presented in detail in appendix 2.

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(3) The Reagent used may have decomposed or deteriorated badly.

Off colors have frequently been observed. If the reaction produces a muddy brown color, inadequate screening procedures are usually the cause. Occasionally in carrying out the reaction, a transient pink color has been noted. Here it should be recognized that the Gibbs reagent reacts with many materials. A transient pink may be produced, for instance, by the reaction of the reagent with minute amounts of p-thiocresol.

The colors produced by different specific "phenols" reacting with Gibbs reagent vary widely. They include greens, blue greens, blues and purples. Accordingly, when a waste under examination contains a complex mixture of "phenols", it is exceedingly difficult to prepare standards for visual comparison. There appears to be only one reasonably satisfactory solution for this problem - - the adoption of arbitrary spectrophotometric means of comparison.

Most of the "phenols" commonly encountered, when reacted with Gibbs reagent, produce colors which show an absorption maximum at or near 670 millimicrons. Collectively and individually the compounds show, at this wave length, an optical density fairly close to the maximum optical density shown by

the compound. For this reason, the advisory committee for the I.J.C. (4) elected to base phenol estimation on the comparison of optical densities of unknowns with phenol standards at 670 millimicrons.

Several of the procedures previously mentioned (4), (5), (6), include extraction of the dye produced by the Gibbs reaction by n-butyl alcohol. The operation is employed to increase the sensitivity of the reaction. On occasion, workers have been puzzled to find an apparent disappearance of the color when extraction with n-butyl alcohol was made, or in some subsequent step in the handling of the alcohol. This difficulty might have a number of causes. Most frequently, it is caused by acids, present as impurities in the n-butyl alcohol used, or picked up from glassware.

The dyes involved are pH indicators. The blue alkaline form has a much more intense color than the red or orange acid forms. Further, the n-butyl alcohol extract is of lower pH than is the aqueous phase which it contacted. Hence, the inadvertent introduction of small amounts of acid, causing the dye to shift its color, may lead an inexperienced analyst to conclude that the reaction products have suddenly disappeared. Introduction of small amounts of 10% alcoholic KOH will restore the desired blue color if pH changes have caused it to disappear.

Reaction rates of individual phenolic compounds with Gibbs reagent vary widely. Certain phenols respond almost immediately to give the maximum color. Thymol and 1-naphthol, for instance, produce the maximum color very shortly after the addition of the Gibbs reagent. With phenol and a number of other phenolic materials, the reaction proceeds slowly, and a long period is required for development of the maximum color intensity. When it is desired to use the test for the determination of individual phenolic materials, the reaction time may be greatly abbreviated in many cases.

When the reaction is carried out as specified by Ettinger and Ruchhoft (6), the amount of color produced per unit weight of phenolic material is a maximum in the case of phenol. This permits a fairly definite interpretation, i.e., the phenol value found indicates the minimum amount of "phenol" which may be present.

The 4-Aminoantipyrine Test

This test is described by Gottlieb and Marsh (7) as a test for phenolic fungicides. However, it does appear to have a definite usefulness to the sanitary field. Essentially, the test consists of reacting phenolic materials with 4-aminoantipyrine in the presence of potassium ferricyanide in alkaline

solution. The sensitivity of the test is very much dependent upon the "phenol" in question. In general, the reagent is very much less sensitive than is the Gibbs reagent if both chemicals react with the phenol in question.

We have had rather limited experience with the test. However, it does not seem to be capable of a high degree of precision when applied to the simpler phenols because the background color shows some tendency to drift.

The test, because of its simplicity and rapidity, should be useful in exploratory field surveys. In the case of certain of the "phenols", a reaction can be obtained with this procedure, whereas the Gibbs test or bromination will not respond. In particular, materials such as pentachlorophenol, trichlorophenol and other highly halogenated phenols, which will not respond to the Gibbs reagent, are reported to react with the 4-aminoantipyrine.

SUMMARY

(1) Analytical procedures for the determination of phenolic materials in industrial wastes consist of the application of a preliminary screening procedure, followed by a measuring reaction.

(2) A satisfactory screening procedure should retain all the desired phenolic constituents. At the same time it should screen out all materials initially present which would give either a positive or a negative interference in the application of the measuring reaction.

(3) Screening procedures consist of the application of one or more of the following operations: (a) distillation, (b) alkaline evaporation, (c) treatment with a precipitant for interfering materials, (d) extractive separations. In general, adequate screening procedures for complex industrial wastes will include both a distillation and an extractive separation. Because in practise alkaline evaporation frequently results in the loss of some phenol, this step should be applied with caution only after preliminary purification procedures have been carried out.

(4) The use of bromine demand as a measuring technique for the estimation of phenol is discussed. The many requirements

for an adequate screening procedure to use ahead of this type of procedure are pointed out. The effects of small variations in the procedure on the magnitude of the results obtained are outlined and the need for close empiricalism in the bromination technique has been emphasized. The amounts of bromine which react with certain phenols under the conditions stipulated by R. D. Scott's procedure (3) are presented.

(5) Some of the causes of difficulty in the use of various modifications of the Gibbs procedure have been discussed. These stem from the following characteristics of the reaction or the products of the reaction:

(a) The reaction takes place satisfactorily in a limited pH range.

(b) The Gibbs reagent reacts with many materials beside phenols to give "off colors".

(c) The materials produced by the reaction may be affected by various oxidants or reductants.

(d) The materials produced by the reaction are also indicators of pH change.

(6) The interpretation of "phenol" tests on industrial wastes is considered at length. When either the bromination test or the Gibbs test is carried out according to specified procedures, the amount of "phenol" reported corresponds

to the <u>minimum</u> amount of phenol which may be present.

(7) The use of 4-aminoantipyrine as a test reagent for phenols has been discussed briefly. This test has the advantage of great rapidity and further responds to certain phenols which cannot be determined by the use of Gibbs reagent or bromination. The disadvantages of the test in the case of the simpler phenols are also considered briefly.

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Appendix I.

Tentative Procedure for the Determination of Phenol in By-Product Coke Wastes.

I. Preliminary Screening Procedure.

1. To 250 ml. of sample, add 2 ml. of 10% $CuSO_4$ solution and bring the pH of the mixture below 4.0 by the addition of 10% phosphoric acid solution. Put the mixture in an all glass distillation apparatus. Distill over approximately 200 ml. Halt the distillation, and allow the distilling flask to cool until boiling ceases. Add 50 ml. of distilled water to the distilling flask. Resume the distillation and continue until a total of 250 ml. has been distilled over.
2. Acidify the distillate with 1 ml. of 10% phosphoric acid solution and add 5 ml. of 10% $CuSO_4$ solution. Transfer the mixture to a separatory funnel containing 75 grams of fine grained sodium chloride. Make 3 serial extractions with c.p. chloroform. On the first extraction, shake the mixture until the salt has completely dissolved. Use 50 ml. of chloroform per extraction. Collect the chloroform extract, discarding the aqueous phase after the third extraction. Combine the chloroform extract. Extract the chloroform twice

with 75 ml. portions of 0.1 N sodium hydroxide. Discard the extracted chloroform. Combine the alkaline extract and make it up to 250 ml. using a volumetric flask.

II. Estimation of "Phenol".

The purified phenol solution is examined for phenol as described by R.D. Scott, Ind. Eng. Chem., Anal. Ed. 3, 67 (1931).

Appendix II.

Determination of Phenol

(Reprinted from "Analytical Methods for Boundary Waters Quality Control", Board of Technical Advisers, International Joint Commission, April 1949, Public Health Service Mimeo.)

1. Sample Preservation and Handling Prior to Analysis. At the time the sample is collected, a 10% solution of $CuSO_4 \cdot 5H_2O$ shall be added at the rate of 10 ml. per liter of sample. Analysis of the sample as described below should be started the day the sample is collected, and in no case shall a sample be held in excess of 24 hours before analysis is begun.

2. Scope of Method.

   The procedure given is specifically designed to remove a multitude of interfering substances which may be present in industrial wastes and for this reason is more complicated than would be required for normal aerobic stream samples.

Materials -

(1) Distilled water - phenol and chlorine free.

(2) Borate buffer.

   3.1 g boric acid (H_3BO_3).

   3.5 g KCl

   32 ml. of 1 N. NaOH

Make up to 1 liter with distilled water. Make up 5 ml. of the above buffer solution to 100 ml. with freshly distilled water, and if the pH is not 9.4, add further caustic until 5 ml. of the buffer solution produces a pH of 9.4 when made up to 100 ml. with distilled water.

(3) Gibbs' reagent: (Alcoholic Concentrate).

Dissolve 0.2 g. of 2,6-dibromquinonechlorimide in 50 ml. of ethyl alcohol. Filter out any residue. If refrigerated, this reagent may be used for periods up to one week.

(4) Gibbs' reagent (Aqueous Dilute Solution).

Dilute 4.5 ml. of the alcoholic concentrate to 100 ml., use within 30 minutes, and preferably immediately.

Special Apparatus

(1) Spectrophotometer or Electrophotometer capable of accommodating a cell of at least 5 cm. length which can be satisfactorily filled with 60 cc. of liquid or less. Further, wave length selection of the light used shall be such that the instrument can operate with a wave length of 670 millimicrons and a slit width or wave length spread of not over 15 millimicrons, preferably of 2 millimicrons or less.

(2) Distillation Apparatus.

1 liter Pyrex No. 3360 distilling apparatus with Graham condenser, or equivalent.

Details of Procedure

(1) To 606 cc. of the sample treated with copper sulphate solution, add 1.5 ml. of 10% H_3PO_4.

Check the pH of the sample, and if it is not below 4.0, add sufficient 10% H_3PO_4 to bring the pH below 4.0.

Using an all-glass still and condenser, such as a 1 liter Pyrex No. 3360 distilling flask with Graham condenser, the sample is distilled. 550 cc. are distilled over rapidly. The distillation is then halted and 50 cc. of chlorine and phenol free distilled water are added to the residue in the distilling flask. A further 50 cc. are distilled over more slowly in order to avoid cracking the distilling flask. The total distillate collected is 600 cc. Acidify the distillate with 1 cc. of concentrated H_3PO_4 and add 5 cc. of 10% $CuSO_4$. Place the sample in a 1 liter separatory funnel. Add 125 cc. of C.P., ACS diethyl ether and shake the sample with the ether thoroughly. Separate the sample from the ether layer and make a second extraction of the sample with 125 ml. of ether, again separating the ether layer and combining it with the ether separated after the first extraction. Discard the water phase. Extract the ether sequentially with two 75 cc. portions of N/10 NaOH. Combine both alkaline extracts of the ether, discarding the ether after the

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second extraction. Cautiously heat the alkaline extract under a hood, using an electric heater. Bring to a boil and boil off sufficient liquor to reduce the volume to approximately 100 cc. Add 2 cc. of concentrated phosphoric acid, 5 cc. of 10% $CuSO_4$ and make up the volume to 300 cc. Using the type of distillation apparatus previously described, distill over 250 cc. at a rapid rate. Halt the distillation. Add 50 cc. of distilled water. Distill over a further 50 cc. at a slower rate to bring the total volume collected up to 300 cc.

Control portions should be prepared as follows:

(a) Blank - 300 cc. of distilled water.

(b) Phenol control - 5 cc. of a 1000 p.p.m. solution of phenol in water are diluted up to 1 liter. 10 cc. of this dilution are made up to 1 liter to yield a 50 p.p.b. solution (0.05 p.p.m.). To 600 cc. of this solution, 6 cc. of $CuSO_4$ are added, and this control portion is treated exactly as is a sample under examination.

Place all samples, the phenol control and the blank control in glass stoppered vessels of similar shape. Adjust the temperature of all portions so that all portions show less than 1° C. difference from the

temperature of the phenol control, and the temperature is under 28° C. Add 15 ml. of borate buffer to each portion under examination and mix well by shaking. Add to each portion 5 ml. of freshly prepared dilute Gibbs' reagent (aqueous dilute solution) and shake well immediately. Allow the samples to stand from 6 to 24 hours under identical temperature conditions, below 28° C. To each portion add 75 ml. of n-butyl alcohol and shake thoroughly. Separate the water and n-butyl alcohol layers using 500 ml. separatory funnels. Filter the alcoholic dye extract through a 18.5 cm. fluted paper.* After the paper is drained completely, wash once with a measured 5 ml. portion of n-butyl alcohol, combining the two portions of the filtrate. Make all of the alcoholic extract up to exactly 60 ml. with n-butyl alcohol and mix each portion by gentle swirling. The optical density of all portions, including the control phenol sample, are now determined using a spectrophotometer or an electrophotometer, the sample being examined at a wave length of 670 millimicrons, with cells of not less than 5 cm. in length of light path, and a slit opening

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* Filter paper should be washed with distilled water, made slightly alkaline with ammonium hydroxide, before filtration of the n-butyl extract.

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of not more than 15 millimicrons, and preferably not more than 1 millimicron. The samples and phenol standard are compared against the control blank as a reference.

Calculation -

$$\text{phenol, p.p.b.} = \frac{50. \text{ Optical Density of Sample (670 millimicrons)}}{\text{Optical Density of Control (670 millimicrons) Phenol}}$$

If the optical density of the sample is greater than that of the control phenol sample, phenol shall be reported as "over 50 p.p.b."